

What is claimed is:

1 1. A radiolabeled immunotoxin comprising a toxic domain,  
2 a targeting domain, and at least one radionuclide atom,  
3 wherein the targeting domain is a single-chain Fv (sFv)  
4 antibody fragment that binds to a target molecule on a target  
5 cell, wherein the target molecule is not an ε chain of a T  
6 cell CD3 complex.

1 2. The radiolabeled immunotoxin of claim 1, wherein the  
2 toxic domain is a toxic polypeptide selected from the group  
3 consisting of: (a) ricin, (b) *Pseudomonas* exotoxin (PE); (c)  
4 bryodin; (d) gelonin; (e) α-sarcin; (f) aspergillin; (g)  
5 restrictocin; (h) angiogenin; (i) saporin; (j) abrin;  
6 (k) pokeweed antiviral protein (PAP); (l) a ribonuclease; (m) a  
7 pro-apoptotic polypeptide; and (n) a functional fragment of  
8 any of (a)-(m).

1 3. The radiolabeled immunotoxin of claim 1, wherein the  
2 toxic domain is diphtheria toxin (DT) or a functional fragment  
3 thereof.

1 4. The radiolabeled immunotoxin of claim 3, wherein the  
2 toxic domain comprises amino acids 1-389 of DT.

2 5. The radiolabeled immunotoxin of claim 1, wherein the  
3 target cell is a cancer cell.

1 6. The radiolabeled immunotoxin of claim 5, wherein the  
2 cancer cell is selected from the group consisting of a neural  
3 tissue cancer cell, a melanoma cell, a breast cancer cell, a

4 lung cancer cell, a gastrointestinal cancer cell, an ovarian  
5 cancer cell, a testicular cancer cell, a lung cancer cell, a  
6 prostate cancer cell, a cervical cancer cell, a bladder cancer  
7 cell, a vaginal cancer cell, a liver cancer cell, a renal  
8 cancer cell, a bone cancer cell, and a vascular tissue cancer  
9 cell.

1 7. The radiolabeled immunotoxin of claim 5, wherein the  
2 target molecule is Her-2/neu.

1 8. The radiolabeled immunotoxin of claim 5, wherein the  
2 target molecule is selected from the group consisting of a  
3 mucin molecule, carcinoembryonic antigen (CEA), prostate-  
4 specific antigen (PSA), folate binding receptor, A33 alpha  
5 fetoprotein, CA-125 glycoprotein, colon-specific antigen p,  
6 ferritin, p-glycoprotein, G250, OA3, PEM glycoprotein, L6  
7 antigen, 19-9, P97, placental alkaline phosphatase, 7E11-C5,  
8 17-1A, TAG-72, 40 kDa glycoprotein, URO-8, a tyrosinase, an  
9 interleukin- (IL-)2 receptor polypeptide, an IL-3 receptor  
10 polypeptide, an IL-13 receptor polypeptide, an IL-4 receptor  
11 polypeptide, a vascular endothelial growth factor (VEGF)  
12 receptor, a granulocyte macrophage-colony stimulating factor  
13 (GM-CSF) receptor polypeptide, an epidermal growth factor  
14 (EGF) receptor polypeptide, an insulin receptor polypeptide,  
15 an insulin-like growth factor receptor polypeptide,  
16 transferrin receptor, estrogen receptor, a T cell receptor  
17 (TCR)  $\alpha$ -chain, a TCR  $\beta$ -chain, a CD4 polypeptide, a CD8  
18 polypeptide, a CD7 polypeptide, a B cell immunoglobulin (Ig)  
19 heavy chain, a B cell Ig light chain, a CD19 polypeptide, a  
20 CD20 polypeptide, a CD22 polypeptide, a MAGE polypeptide, a

21 BAGE polypeptide, a GAGE polypeptide, a RAGE polypeptide, a  
22 PRAME polypeptide, and a GnTV polypeptide.

1 9. The radiolabeled immunotoxin of claim 1, wherein the  
2 radionuclide is selected from the group consisting of  $^{90}\text{Y}$ ,  
3  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$ ,  $^{213}\text{Bi}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{211}\text{At}$ ,  
4  $^{32}\text{P}$ ,  $^{177}\text{Lu}$ ,  $^{47}\text{Sc}$ ,  $^{105}\text{Rh}$ ,  $^{109}\text{Pd}$ ,  $^{153}\text{Sm}$ ,  $^{199}\text{Au}$ ,  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$ ,  $^{124}\text{I}$ ,  $^{18}\text{F}$ ,  
5  $^{11}\text{C}$ ,  $^{198}\text{Au}$ ,  $^{75}\text{Br}$ ,  $^{76}\text{Br}$ ,  $^{77}\text{Br}$ ,  $^{13}\text{N}$ ,  $^{34\text{m}}\text{Cl}$ ,  $^{38}\text{Cl}$ ,  $^{52\text{m}}\text{Mn}$ ,  $^{55}\text{Co}$ ,  $^{62}\text{Cu}$ ,  
6  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{76}\text{As}$ ,  $^{72}\text{Se}$ ,  $^{73}\text{Se}$ , and  $^{75}\text{Se}$ .

1 10. A radiolabeled multimeric immunotoxin comprising:  
2 (a) at least two monomers; and  
3 (b) at least one radionuclide atom,  
4 wherein each monomer comprises a targeting domain  
5 and a toxic domain and is physically associated with the other  
6 monomers,  
7 wherein the targeting domain binds to a target  
8 molecule on a target cell.

1 11. The radiolabeled multimeric immunotoxin of claim 10,  
2 wherein each of said monomers further comprises one or more  
3 coupling moieties and the physical association of the monomer  
4 is by at least one of the one or more coupling moieties.

1 12. The radiolabeled multimeric immunotoxin of claim 11,  
2 wherein the coupling moiety is a terminal moiety.

1 13. The radiolabeled multimeric immunotoxin of claim 12,  
2 wherein the terminal moiety is a C-terminal moiety.

1 14. The radiolabeled multimeric immunotoxin of claim 11,  
2 wherein the one or more coupling moieties are cysteine  
3 residues.

1 15. The radiolabeled multimeric immunotoxin of claim 11,  
2 wherein at least one of the one or more coupling moieties is a  
3 heterologous coupling moiety.

1 16. The radiolabeled multimeric immunotoxin of claim 10,  
2 wherein each of the monomers comprises the same amino acid  
3 sequence.

1 17. An *in vitro* method of killing a target cell, the  
2 method comprising culturing the target cell with the  
3 radiolabeled immunotoxin of claim 1.

1 18. A method comprising:  
2 (a) identifying a subject suspected of having a  
3 pathogenic cell disease; and  
4 (b) administering to the subject a radiolabeled  
5 immunotoxin comprising a toxic domain, a targeting domain, and  
6 at least one radionuclide atom, wherein the targeting domain  
7 is a sFv antibody fragment that binds to a target molecule on  
8 a target cell in the subject.

1 19. The method of claim 18, wherein the toxic domain is  
2 a toxic polypeptide selected from the group consisting of: (a)  
3 ricin, (b) *Pseudomonas* exotoxin (PE); (c) bryodin; (d)  
4 gelonin; (e)  $\alpha$ -sarcin; (f) aspergillin; (g) restrictocin; (h)  
5 angiogenin; (i) saporin; (j) abrin; (k) pokeweed antiviral  
6 protein (PAP); (l) a ribonuclease; (m) a pro-apoptotic  
7 polypeptide, and (n) a functional fragment of any of (a)-(m).

1 20. The method of claim 18, wherein the toxic domain is  
2 diphtheria toxin (DT) or a functional fragment thereof.

1 21. The method of claim 20, wherein the functional  
2 fragment comprises amino acids 1-389 of DT.

1 22. The method of claim 18, wherein the target cell is a  
2 cancer cell.

1 23. The method of claim 22, wherein the cancer cell is  
2 selected from the group consisting of a neural tissue cancer  
3 cell, a melanoma cell, a breast cancer cell, a lung cancer  
4 cell, a gastrointestinal cancer cell, an ovarian cancer cell,  
5 a testicular cancer cell, a lung cancer cell, a prostate  
6 cancer cell, a cervical cancer cell, a bladder cancer cell, a  
7 vaginal cancer cell, a liver cancer cell, a renal cancer cell,  
8 a bone cancer cell, and a vascular tissue cancer cell.

1 24. The method of claim 22, wherein the target molecule  
2 is Her-2/neu.

1 25. The method of claim 22, wherein the target molecule  
2 is selected from the group consisting of a mucin molecule,  
3 CEA, PSA, folate binding receptor, A33 alpha fetoprotein, CA-  
4 125 glycoprotein, colon-specific antigen p, ferritin, p-  
5 glycoprotein, G250, OA3, PEM glycoprotein, L6 antigen, 19-9,  
6 P97, placental alkaline phosphatase, 7E11-C5, 17-1A, TAG-72,  
7 40 kDa glycoprotein, URO-8, a tyrosinase, an interleukin-  
8 (IL-)2 receptor polypeptide, an IL-3 receptor polypeptide, an  
9 IL-13 receptor polypeptide, an IL-4 receptor polypeptide, a  
10 VEGF receptor, a GM-CSF receptor polypeptide, an EGF receptor

11 polypeptide, an insulin receptor polypeptide, an insulin-like  
12 growth factor receptor polypeptide, transferrin receptor,  
13 estrogen receptor, a T cell receptor (TCR)  $\alpha$ -chain, a TCR  $\beta$ -  
14 chain, a CD4 polypeptide, a CD8 polypeptide, a CD7  
15 polypeptide, a B cell Ig heavy chain, a B cell Ig light chain,  
16 a CD19 polypeptide, a CD20 polypeptide, a CD22 polypeptide, a  
17 MAGE polypeptide, a BAGE polypeptide, a GAGE polypeptide, a  
18 RAGE polypeptide, a PRAME polypeptide, and a GnTV polypeptide.

1 26. The method of claim 18, wherein the method is a  
2 method of killing a target cell in the subject.

1 27. The method of claim 26, wherein the radionuclide is  
2 selected from the group consisting of  $^{90}\text{Y}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{64}\text{Cu}$ ,  
3  $^{67}\text{Cu}$ ,  $^{212}\text{Pb}$ ,  $^{212}\text{Bi}$ ,  $^{213}\text{Bi}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{211}\text{At}$ ,  $^{32}\text{P}$ ,  $^{177}\text{Lu}$ ,  $^{47}\text{Sc}$ ,  
4  $^{105}\text{Rh}$ ,  $^{109}\text{Pd}$ ,  $^{153}\text{Sm}$ , and  $^{199}\text{Au}$ .

1 28. The method of claim 18, wherein the method is an  
2 imaging method.

1 29. The method of claim 28, wherein the radionuclide is  
2 selected from the group consisting of  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{64}\text{Cu}$ ,  $^{67}\text{Cu}$ ,  
3  $^{212}\text{Bi}$ ,  $^{123}\text{I}$ ,  $^{131}\text{I}$ ,  $^{211}\text{At}$ ,  $^{177}\text{Lu}$ ,  $^{47}\text{Sc}$ ,  $^{105}\text{Rh}$ ,  $^{109}\text{Pd}$ ,  $^{153}\text{Sm}$ ,  $^{199}\text{Au}$ ,  $^{99\text{m}}\text{Tc}$ ,  
4  $^{111}\text{In}$ ,  $^{124}\text{I}$ ,  $^{18}\text{F}$ ,  $^{11}\text{C}$ ,  $^{198}\text{Au}$ ,  $^{75}\text{Br}$ ,  $^{76}\text{Br}$ ,  $^{77}\text{Br}$ ,  $^{13}\text{N}$ ,  $^{34\text{m}}\text{Cl}$ ,  $^{38}\text{Cl}$ ,  $^{52\text{m}}\text{Mn}$ ,  
5  $^{55}\text{Co}$ ,  $^{62}\text{Cu}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{76}\text{As}$ ,  $^{72}\text{Se}$ ,  $^{73}\text{Se}$ , and  $^{75}\text{Se}$ .

1 30. A method of making a radiolabeled immunotoxin, the  
2 method comprising:

3 (a) providing a cell comprising a vector containing  
4 a nucleic acid sequence encoding a protein, the nucleic acid  
5 sequence being operably linked to a transcriptional regulatory  
6 element (TRE);

7                   (b) culturing the cell;  
8                   (c) extracting the protein from the culture; and  
9                   (d) attaching at least one radionuclide atom to the  
10 protein,

11                   wherein the protein comprises a toxic domain and a  
12 targeting domain,

13                   wherein the targeting domain is a sFv antibody  
14 fragment that binds to a target molecule on a target cell,  
15 wherein the target molecule is not a polypeptide of the CD3  
16 complex.

1       31.       A method of making a radiolabeled multimeric  
2 immunotoxin, the method comprising:

3                   (a) providing one or more cells, each of the cells  
4 comprising a nucleic acid sequence encoding a monomer with a  
5 different amino acid sequence, wherein the nucleic acid  
6 sequence is operably linked to a TRE;

7                   (b) separately culturing each of the one or more  
8 cells;

9                   (c) extracting the monomer from each of the  
10 cultures;

11                   (d) exposing the monomers to conditions which allow  
12 multimerization of the monomers to form a multimer comprising  
13 at least two monomers; and

14                   (e) attaching at least one radionuclide atom to the  
15 multimer,

16                   wherein each monomer comprises a targeting domain  
17 and a toxic domain,

18                   wherein the targeting domain binds to a target  
19 molecule on a target cell.

1 32. A method of making a radiolabeled immunotoxin, the  
2 method comprising:

3 (a) providing a protein comprising a toxic domain  
4 and a targeting domain; and  
5 (b) attaching at least one radionuclide atom to the  
6 protein,  
7 wherein the targeting domain is a sFv antibody  
8 fragment that binds to a target molecule on a target cell,  
9 wherein the target molecule is not an ε chain of a T cell CD3  
10 complex.

1 33. A method of making a radiolabeled multimeric  
2 immunotoxin, the method comprising:

3 (a) providing a multimeric protein; and  
4 (b) attaching at least one radionuclide atom to the  
5 multimeric protein;  
6 wherein the multimeric protein comprises at least  
7 two monomers,  
8 wherein each monomer comprises a targeting domain  
9 and a toxic domain and is physically associated with the other  
10 monomers,  
11 wherein the targeting domain binds to a target  
12 molecule on a target cell.

1 34. The radiolabeled multimeric immunotoxin of claim 10,  
2 wherein the targeting domain is an antibody fragment.

1 35. The radiolabeled multimeric immunotoxin of claim 34,  
2 wherein the antibody fragment is a sFv.

1 36. The radiolabeled multimeric immunotoxin of claim 34,  
2 wherein the antibody fragment binds to a target molecule on a  
3 T cell.

1 37. The radiolabeled multimeric immunotoxin of claim 34,  
2 wherein the target molecule is a CD3 polypeptide.

1 38. The radiolabeled multimeric immunotoxin of claim 10,  
2 wherein the targeting domain is a targeting polypeptide  
3 selected from the group consisting of: (a) a cytokine; (b) a  
4 ligand for a cell adhesion receptor; (c) a ligand for a signal  
5 transduction receptor; (d) a hormone; (e) a molecule that  
6 binds to a death domain family molecule; (f) an antigen; and  
7 (g) a functional fragment of any of (a) - (f).

1 39. The radiolabeled immunotoxin of claim 1, further  
2 comprising one or more additional targeting domains.

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